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1 **Courtship and mating behaviour of manta rays *Mobula alfredi* and *M.***
2 ***birostris* in the Maldives**

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8 Running head: COURTSHIP AND MATING BEHAVIOUR OF MANTA RAYS

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ABSTRACT

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Manta rays *Mobula alfredi* and *M. birostris* are among the most conspicuous and charismatic of the elasmobranchs, however their courtship and mating behaviour is rarely observed. Although the mating stages of manta ray reproduction have been described, the full detail of their elaborate courtship has not. The aim of this fourteen year study was to elucidate the entire courtship and mating behaviour of both manta ray species using behavioural observations, video and photographic records. From 2003 through 2016, over 11,000 surveys were undertaken at known manta ray aggregation sites in the Maldives to record any observed manta ray reproductive activity. A total of 47,591 photo-ID sightings of 4,247 individual *M. alfredi* and 229 photo-ID sightings of 226 individual *M. birostris* were recorded at 22 atolls and across 265 different sites. Courtship activity was observed on 206 surveys at 30 different sites. A total of 229 courtship events were recorded, with 90% (n=205) of them occurring at cleaning sites. The observed courtship activity was categorised into seven distinct stages which are described in detail: (1) initiation, (2) endurance, (3) evasion, (4) pre-copulatory positioning, (5) copulation, (6) post-copulatory holding, and (7) separation. Photographs provide the first scientific record of the entirety of manta ray courtship and mating. Both *M. alfredi* and *M. birostris* appear to engage in the same elaborate courtship rituals, exhibiting the same behaviours during all stages of the courtship and mating process.

Key words: cleaning stations, copulation, courtship trains, mate choice, reproductive behaviour

INTRODUCTION

43

44 Although much is known about the reproductive anatomy and different modes of
45 elasmobranch reproduction (Wourms, 1977; Dodd, 1983; Gilmore, 1993; Wourms & Demski,
46 1993; Conrath & Musick, 2012), shark and ray reproductive behaviour is poorly understood,
47 mainly due to the difficulties of observing natural behaviour in free-living animals and the
48 artificial nature of studies in captivity (Whitney et al., 2004). Most recently, Pratt and Carrier
49 (2005) summarised the published literature on elasmobranch reproductive behaviour, but
50 their work represented just a small fraction of the total species pool. In their study, many
51 reports came from captive animals and little photographic documentation was provided.

52 Manta rays are highly conspicuous and charismatic elasmobranchs. Until 2017 these
53 zooplanktivorous rays were separated from other mobulids in their own genus: *Manta*
54 (Bancroft, 1829). However, the Mobulidae family is now considered monogenetic, comprising
55 nine species in the Genus *Mobula* (Rafinesque, 1810; White et al., 2017). In 2009, the
56 monospecific *Manta* was split into the two species of manta ray currently recognised
57 (Marshall et al., 2009): the reef manta ray *Mobula alfredi* (Krefft, 1868) and the oceanic manta
58 ray *Mobula birostris* (Walbaum, 1792). *Mobula alfredi* is widely distributed throughout the
59 tropical and sub-tropical waters of the Pacific and Indian Oceans, although populations
60 appear to be highly fragmented (Kashiwagi et al., 2011; Couturier et al., 2012); most likely
61 due to resource and habitat requirements (Anderson et al., 2011a). *Mobula alfredi* frequent
62 the coastal reefs of continents and remote oceanic islands (Kashiwagi et al., 2011; Marshall
63 et al., 2011a), but also venture offshore into the mesopelagic zone (Braun et al., 2014; Jaine
64 et al., 2014). *Mobula birostris* is distributed throughout the tropics, and within waters up to
65 41° of latitude (Kashiwagi et al., 2011). It is thought that *M. birostris* spend the majority of

66 their time offshore, but come into shallower regions along productive coastlines with regular
67 upwellings (Marshall et al., 2011b; Stewart et al., 2016a). *Mobula birostris* also aggregate
68 around oceanic island groups and offshore pinnacles, seamounts and submarine ridge
69 systems, where they are known to engage in courtship (Compagno & Last, 1999; Yano et al.,
70 1999; Rubin, 2002; Marshall et al., 2011b; Stewart et al., 2016b).

71 Manta rays are ovoviviparous matrotrophs (Wourms, 1977; Dulvy & Reynolds, 1997) and
72 like all elasmobranchs, employ internal fertilization (Conrath & Musick, 2012). The gestation
73 time of *M. alfredi* is reported to be one year (Marshall & Bennett, 2010; Okinawa Churaumi
74 Aquarium, 2010; Deakos, 2011; Stevens, 2016), but remains unknown for *M. birostris*.
75 Reproductive cycles often include resting periods, with biennial reproduction reported as the
76 norm for individual *M. alfredi* within populations in Hawaii and Mozambique (Marshall &
77 Bennett, 2010; Deakos, 2011). Inter-birth intervals of several years or more are common in
78 matrotrophs which also invest heavily in post parturition parental care, such as Sumatran
79 orangutans *Pongo pygmaeus abelii*, capuchins *Cebus capucinus*, African and Asian elephants
80 *Loxodonta africana* and *Elephas maximus*, and bottlenose dolphins *Tursiops sp.* (Lee & Moss,
81 1986; Fedigan & Rose, 1995; Mann et al., 2000; van Noordwijk & van Schaik, 2005; Robinson
82 et al., 2012), but are less common in species which do not. Amongst species which do not
83 undertake post parturition parental care, biennial and triennial reproductive cycles have only
84 been reported in reptiles (Cree & Guillette, 1995; Ibarra-Guerrero & Cussac, 1996; Sever et al.,
85 2000) and elasmobranchs (Colonello et al., 2006; Whitney & Crow, 2006; Castro, 2009). As a
86 general point, rest periods between reproduction are thought to occur to allow females to
87 recuperate energy reserves (Catry et al., 2006; Trinnie et al., 2012). In manta ray reproduction,
88 females normally give birth to a single, large pup (Coles, 1916; Beebe & Tee-Van, 1941;
89 Bigelow & Schroeder, 1953), although rare cases of twins have been recorded (Marshall &

90 Bennett, 2010), with size at birth ranging from 130 – 190 cm in disc width (Marshall & Bennett,
91 2010; Okinawa Churaumi Aquarium, 2010).

92 Manta ray reproductive behaviour in the wild has rarely been observed and virtually all
93 documentation that exists is for *M. alfredi*, with just one record of mating for *M. birostris*. This
94 was recorded off the Ogasawara Islands, Japan in 1997 and describes a female copulating with
95 two different males on the same day (Yano et al., 1999). For *M. alfredi*, most courtship and
96 mating reported from the wild has been at manta feeding and cleaning sites (Marshall &
97 Bennett, 2010; Deakos, 2011). The mating events number just five (Marshall & Bennett, 2010)
98 and all described females mating once, with one male.

99 Field observations in Japan, Mozambique and Hawaii indicate the same complex mating
100 process occur in both manta ray species, and that this involves a five-step sequence of (1)
101 chasing, (2) biting, (3) copulating, (4) post-copulation holding and (5) separation (Yano et al.,
102 1999; Marshall & Bennett, 2010; Deakos, 2011). Copulation occurs when the female slows to
103 allow a pursuing male to position himself directly on top of her dorsal surface. At this point
104 the male slides his mouth down the side of the female's body, nearly always her left, to the
105 tip of her pectoral fin, before taking about a metre of this fin into his mouth. The male then
106 bites down hard to gain leverage on the female's body, twisting underneath her so that the
107 female's ventral surface is in alignment with his, enabling him to insert a clasper into her
108 cloacal opening before releasing his seminal fluid (Yano et al., 1999; Marshall & Bennett,
109 2010).

110 Although observations of actual copulation are extremely rare, pre-copulatory chasing has
111 been more commonly observed, especially in *M. alfredi*, where multiple escorting males
112 pursue a single, fast swimming female in what is commonly termed a 'mating train' (Marshall
113 & Bennett, 2010; Deakos, 2011). This behaviour, known as 'female recruitment runs' appears

114 to be the basis of pre-copulatory mate choice by females and can last for hours (Whitney et
115 al., 2004; Deakos, 2011). However, as it does not always result in mating (Stevens, 2016), and
116 appears to be driven primarily by female mate choice, this behaviour hereafter is referred to
117 as a 'courtship train'.

118 During courtship trains, the female initiates high speed flips, turns and somersaults, while
119 pursuing males mimic her evasive manoeuvres (Marshall & Bennett, 2010; Deakos, 2011).
120 Because several different behaviours appear to take place within step one of the courtship
121 classification described by Yano et al. (1999), Marshall and Bennett (2010) proposed this step
122 should be broken down into three subdivisions namely: (1) following or chasing, (2) a complex
123 series of interactive turning and flipping performed by both the female and her suitors, and
124 (3) evasive or avoidance behaviour exhibited by the female.

125 Courtship trains have been observed in several close relatives of manta rays, namely in:
126 flapnose ray *Rhinoptera javanica*, cownose ray *Rhinoptera bonasus*, spotted eagle ray
127 *Aetobatus narinari*, spinetail devil ray *Mobula mobular* and sicklefin devil ray *Mobula*
128 *tarapacana* (Tricas, 1980; Uchida et al., 1990; pers. obs.). Whitetip reef sharks *Triaenodon*
129 *obesus* and nurse sharks *Ginglymostoma cirratum*, also display the 'mating avoidance' shown
130 in manta rays whereby a female 'arches' her body during attempted copulation by males to
131 keep her cloaca out of their reach (Pratt & Carrier, 2001; Whitney et al., 2004).

132 There are also parallels between the courtship trains of manta rays and the tending
133 behaviour undertaken by ungulates, where a male will associate with an oestrous female until
134 he either copulates with her or is displaced by another male (Vos et al., 1967; Kucera, 1978;
135 Wolff, 1998; Mysterud et al., 2004; Byers et al., 2005). In marine mammals similar behaviour
136 has been observed in humpback whales *Megaptera novaeangliae* (Tyack & Whitehead, 1982;
137 Baker & Herman, 1984; Spitz et al., 2002; Herman et al., 2007).

138 While the major stages of manta ray mating have already been described, this study
139 provides new detail about the process; the aim being to elucidate the entire courtship and
140 mating behaviour of both manta ray species using behavioural observations backed up by
141 video and photographic records.

142

143

METHODS

144 STUDY AREA AND PERIOD

145 Field research was undertaken in the Maldives in the Indian Ocean, where the world's
146 largest known population of *M. alfredi* (Kitchen-Wheeler et al., 2011; Stevens, 2016) occurs
147 and where *M. birostris* is known from several locations where remote seamounts rise from
148 extremely deep water (Kashiwagi et al., 2011; Stevens, 2016). The 26 coral atolls which form
149 the Maldives archipelago extend from 7° north down 870 km to half a degree south of the
150 equator (Fig. 1). During a fourteen year study from 2003 through to the end of 2016, 11,088
151 surveys throughout the Maldives were undertaken at known aggregation sites for *M. alfredi*
152 and *M. birostris* to observe and photographically record their predominant behaviours and to
153 specifically document activity related to courtship and reproduction. At each site surveyed,
154 the predominant behaviour of all observed manta rays was recorded. In total 64 (24%) of the
155 sites surveyed were considered to be primarily used as cleaning sites by the manta rays (Losey
156 Jr, 1972; Côté, 2000; O'Shea et al., 2010; Jaine et al. 2012), 96 (36%) as feeding sites (Dewar
157 et al., 2008), and 105 (40%) sites where manta rays mostly travelled through the area.

158

159 SAMPLING PROTOCOL

160 In the Maldives, manta rays are accustomed to interacting with tourist divers and
161 snorkellers at aggregation sites where the rays predictably gather at certain times of the year
162 to feed, clean and socialise (Anderson et al., 2011b; Stevens, 2016). A typical survey during
163 this study entailed diving or freediving at one of these aggregation sites, where close
164 encounters with the unperturbed rays easily allowed photo-ID images to be taken and
165 observations recorded of the individuals present (Marshall & Pierce, 2012; Stevens, 2016).
166 Manta ray surveys were performed from either a dedicated research vessel or commercial
167 diving vessels. Surveys were performed at different times of day throughout all months of the
168 year. SCUBA surveys lasted on average 60 minutes and ranged to a maximum depth of 30
169 metres. Freediving surveys lasted on average 120 minutes. The first author, or a trained staff
170 member or volunteer from the Manta Trust (www.mantatrust.org), conducted the surveys
171 (Stevens, 2016).

172 When a manta ray was encountered, it was photographed and/or videoed and its: (1)
173 species, (2) sex, (3) pregnancy status if female, (4) maturity status, and (5) behaviours
174 exhibited were recorded. Behavioural activity was distinguished into: (1) feeding, (2) cleaning,
175 (3) cruising, and (4) courtship. The activity which dominated the encounter was recorded as
176 the primary behaviour. Only courtship behaviour is considered further here.

177 The observed courtship activity was categorised using a methodology developed during
178 this study after initial observations found that the courtship and mating stages proposed by
179 Yano et al (1999) and Marshall and Bennett (2010) did not accurately encompass, or define,
180 all of the behaviours observed. As a result, the following seven distinct courtship stages are
181 identified: (1) initiation, (2) endurance, (3) evasion, (4) pre-copulatory positioning, (5)
182 copulation, (6) post-copulatory holding, and (7) separation (Table I).

183 Given the scarcity of courtship or mating behaviour reported in the literature, and the
184 rarity of observing these events in the wild, an extensive search of the online search engine
185 *YouTube* (www.youtube.com) was performed in an attempt to gather further observational
186 data to supplement this study. This online data was also used to ensure the sequence of
187 behaviours described in this study were consistent across sites and populations. Using
188 different combinations of the key words 'manta', 'mating', 'courtship' and 'copulation' the
189 search engine produced results for ~8,000 videos. All resulting videos containing relevant
190 information were viewed (~150) and the manta ray behaviour exhibited within them recorded
191 using the same protocol as field observations.

192

193

RESULTS

194 In total 47,591 photo-ID sightings of 4,247 individual *M. alfredi* and 229 photo-ID sightings
195 of 226 individual *M. birostris* were made from 22 atolls at 265 sites in the Maldives. Courtship
196 activity was observed on 206 surveys at 30 different sites (Table II). Although it was not
197 possible to identify every individual involved, 420 different *M. alfredi* (143 females and 277
198 males) and six *M. birostris* (two females and four males) were individually identified by their
199 unique ventral spot markings after comparing these to existing databases (Marshall & Pierce,
200 2012; Stevens, 2016). Over 14 years, a total of 229 courtship events were recorded, with 90%
201 (n=205) occurring at cleaning sites. All but two courtship events involved *M. alfredi* (Table III).

202 Using the seven stages of manta ray courtship behaviour described in the methodology,
203 73 instances of initiation (stage 1) were observed, 168 of endurance (stage 2), 40 of evasion
204 (stage 3), two of pre-copulation positioning (stage 4), one of copulation (stage 5), no post-
205 copulation holding (stage 6) and one separation (stage 7). Separation without post-copulation

206 holding (stage 6) occurred because in the one case where copulation was observed the couple
207 separated immediately.

208 Only two courtship events were observed for *M. birostris*; the first involved eight males in
209 a train behind one female; and the second just one male and one female. For *M. alfredi*, of
210 the 73 initiation events only 12 (16%) advanced to the endurance stage of a courtship train.
211 The number of males involved in these varied greatly, with a minimum of only one male
212 chasing a single female, to a maximum of 26 males in the train. The average ratio of males-
213 to-females in a single courtship train was 3:1 (SE \pm 0.2, n = 168), although this rose to 5:1 (SE
214 \pm 0.7, n = 40) if the courtship escalated to the evasion stage. On 12 occasions, two females
215 were involved in a single train (Table III). During these events the second of the two females
216 was usually directly behind the lead female, although their positions in the courtship train
217 sometimes varied during the encounter. Based on their highly distended abdomens, a total
218 of 29 of the females (12% of the total) observed engaging in courtship during the study were
219 near-term pregnant individuals, while a further 23 females (10% of the total) observed
220 engaging in courtship activity had fresh mating wounds.

221 The courtship and mating events recorded in this study have allowed a clearer
222 understanding of manta ray behaviour to emerge, specifically as a result of several
223 noteworthy events amongst the observations. These events are described below in the
224 sequence in which manta ray courtship and mating occurs from specific examples recorded
225 in this study (Table III).

226

227 STAGE ONE (INITIATION)

228 *Event 136: 06/04/2015 – Rasfari North, North Malé Atoll*

229 At 9:57 am while four adult *M. alfredi* were observed to circle the site's cleaning station,
230 an approaching male swam straight towards one of the females being cleaned. Manoeuvring
231 himself directly above her, he unfurled his cephalic fins and placed them onto the female's
232 head (Fig. 2). The female reacted by rapidly raising her body forcefully into the male's ventral
233 surface, physically pushing him backwards. This upward thrust was followed by a flick of one
234 pectoral fin in what appeared an attempt to dislodge the male. This action resulted in the
235 displacement of the male from the female's back and cessation of further courtship.

236

237 STAGES TWO (ENDURANCE) AND THREE (EVASION)

238 *Event 59: 08/11/2008 – Lankan Beyru, North Malé Atoll*

239 The event began at 9:24 am with a repeat of the behaviour described above, except this
240 time the female reacted to the male's presence directly above her by rapidly swimming
241 forwards along the reef. The male followed, attempting to maintain his position on top of the
242 female's back. The ensuing courtship train was observed for several minutes while the pair
243 remained within sight along the reef crest between 5 – 20 metres deep. Periodically the
244 female undertook several forward flips and backward somersaults, while the male, shadowing
245 her movements, remained within one or two metres at all times (Fig. 3). Between flips and
246 somersaults the female swam faster than is usual, and made several quick changes in
247 direction while the male stayed close. During this encounter fresh mating wounds were visible
248 on the tip of the female's left pectoral fin, indicating she had recently mated (Fig. 3 circled).

249

250 *Event 39: 07/11/2007 – Lankan Beyru, North Malé Atoll*

251 This event spanned two dives which were both approximately 60 minutes long. 16 minutes
252 into the first dive at 07:16 am, while observing three adult female *M. alfredi* at the main

253 cleaning station on the reef at 20 metres depth, another female swam rapidly overhead
254 followed by 26 males in a courtship train. Due to the large number of males their trailing line
255 was less delineated than it would be in a more typical chain of several males. Over the next
256 fourteen minutes the courtship train remained within sight and, as in event 59, the female
257 undertook multiple tight turns, forward flips and backward somersaults. Often she would loop
258 back on herself to slot behind the following males, causing apparent confusion, resulting in
259 an unstructured group around the cleaning station. The female also appeared to chase
260 individual males at times, closely following one of the leading males in the courtship train
261 while multiple other pursuing males attempted to position themselves onto her dorsal
262 surface. Each time one of the pursuing males succeeded in getting within touching distance
263 of her back she would perform another series of forward flips, or backward somersaults. After
264 14 minutes the female rapidly swam off followed by the train of males, at which point
265 observations ceased until the next dive (Fig. 4). At 11:44 on the second SCUBA dive, the same
266 female from the previous courtship train again swam along the reef crest at a depth of 15
267 metres, this time pursued by eight males. Six were later identified as present at the earlier
268 courtship event. The courtship train passed quickly and remained in sight for only three
269 minutes. The total time between first and last sighting of this courtship train was four hours
270 and 31 minutes.

271

272 *Event 51: 20/09/2008 – Hanifaru Bay, Baa Atoll*

273 This event took place at 15:26 while the observers were free-diving inside Hanifaru Bay
274 and involved five males and a single near-term pregnant female. The observation lasted two
275 minutes. The pregnant female swam rapidly into the vicinity of the observers, where visibility
276 was only 12 metres, followed by the five males in a courtship train. In ten metres of water the

277 female undertook four tight backwards somersaults while the following males attempted to
278 maintain position close behind, or directly on top of the female's dorsal surface (Fig. 5). After
279 the last somersault the female rapidly swam out of sight followed by all of the males.

280

281 *Event 88: 06/06/2013 – Hurai Faru, Baa Atoll*

282 A courtship event similar to event 50 at Hanifaru Bay occurred several years later at the
283 nearby *M. alfredi* feeding site of Hurai Faru, in which a female was engaged in a courtship
284 train with three males. The female employed similar evasive flipping and somersault
285 behaviour, although in this instance on two occasions she intentionally swam at increased
286 speed within a metre of the freediving observers. On the second of these approaches the
287 female, pursued by the three males, swam directly towards one observer, leaping clear of the
288 water before landing partially on top of the observer, who was filming. The footage can be
289 viewed at: <https://youtu.be/9tpkVjcxqK8>.

290 During this event the female also actively switched from being pursued, to following one
291 of the males for approximately 30 seconds after a series of flipping manoeuvres which
292 resulted in her looping back. Similar female pursuit behaviour was also observed in three
293 other events (Nos. 63, 84 and 105).

294

295 *Event 77: 08/04/2012 – North Point, Fuvahmulah Atoll*

296 During the study only two courtship events involved *M. birostris*. Both occurred at the
297 remote atoll of Fuvahmulah in the far south of the Maldives, a known aggregation site for the
298 species. During this event one large female (disc width ~5 metres) was chased by eight smaller
299 males (average disc width ~4 metres) along the reef crest of the northern tip of the atoll. The
300 observation lasted only a minute, but the behaviour was similar to courtship trains recorded

301 for *M. alfredi*. The second *M. birostris* event (No. 135) also lasted one minute and involved
302 one male chasing a single female along the Southern Spur Reef of the atoll.

303

304 STAGE FOUR (PRE-COPULATION POSITIONING)

305 *Event 2: 06/01/2004 – Rangali Madivaru, Ari Atoll*

306 This event occurred on the shallow reef crest in water three metres deep close to another
307 well-known *M. alfredi* cleaning station frequented by tourists. At 15:20 the sight of a leaping
308 manta ray and the resulting splash alerted observers to the presence of several *M. alfredi* and
309 these were followed by snorkel for the next three minutes. The courtship event involved a
310 lead female and two males. When first spotted, the two males were attempting to position
311 themselves onto the dorsal surface of the female, which remained almost motionless one
312 metre above the reef (Fig. 6a). Both the male's mouths were open as each physically
313 competed to engulf her left pectoral fin (Fig. 6b – d). After 60 seconds one of the males
314 succeeded, whereupon he rotated and flipped his body underneath the female's to align his
315 abdomen against hers (Fig. 6e – h). Throughout this process the female remained motionless,
316 while the other male carried on trying to gain purchase on the female's left pectoral fin, using
317 his head and body to ram the successful male which remained firm (Fig. 6g).

318 The lack of forward swimming motion by the three negatively buoyant manta rays, and the
319 very shallow water, caused all three to sink onto the reef (Fig. 6i). Upon collision the male
320 holding the female released his grasp, allowing her to rapidly swim off with both males in
321 pursuit. Copulation was not seen.

322

323 STAGES FIVE (COPULATION) AND SEVEN (SEPARATION)

324 *Event 1: 19/11/2003 – Lankan Beyru, North Malé Atoll*

325 Observers encountered a single male *M. alfredi* following a female at 10:52 am at 20
326 metres depth on SCUBA. The manta rays were swimming rapidly along the reef crest at
327 approximately 15 metres depth close to the nearby cleaning station where several other
328 manta rays were being cleaned. The female's swimming behaviour was erratic; making tight
329 twists and turns, she swam directly at the observers, passing within less than a metre, while
330 the male manta ray maintained a position less than two metres behind the female at all times.
331 The pair quickly disappeared from view, swimming too fast to be followed. Five minutes later
332 they re-appeared from the direction in which they had departed with the female in the lead.
333 Their swimming speed was now reduced to normal cruising and erratic movements had
334 ceased. When the manta rays were parallel to the observers the male drew closer to the
335 female, positioning himself directly on to her dorsal surface (Fig. 7a). The female reacted by
336 slowly swimming up, at which point the male began to slide his open mouth down the leading
337 edge of her left pectoral fin, using his cephalic fins to guide the tip into his mouth; engulfing
338 one metre, the male then grasped hold of the fin (Fig. 7b – d). The female ceased swimming
339 while the male flipped his body underneath her, abdomen to abdomen in the water column
340 within 10 metres of the surface. The male then inserted a clasper into the female's cloacal
341 opening, while continuing to slowly beat his pectoral fins (Fig. 7e). He made rapid pelvic
342 thrusts which lasted for 10 seconds as the copulating pair slowly spiralled clockwise while
343 sinking. Copulation lasted for approximately 30 seconds before they separated and swam off
344 in different directions. Removal of the male's clasper resulted in a small milky cloud of fluid,
345 presumably sperm, released from the female's cloaca. This action occurred simultaneously
346 with the male releasing his grip on the female's pectoral fin. No post-copulation holding
347 occurred.

348

349 *Supplementary on-line observational footage*

350 An internet search on the video platform *YouTube* revealed ten manta ray courtship and
351 mating event videos which show behaviour rarely observed (Table IV). Footage came from a
352 wide variety of locations and for both species provided rarely seen copulation (Table IV,
353 events one, two and ten). In all three, as in the copulation observed in this study, there was
354 no post-copulatory holding by males, with copulation ceasing simultaneously when the male
355 released his grip on the female's pectoral fin. Event two of the online observations records
356 behaviour very similar to the mass courtship event observed during this study (event 39).
357 Online events three and five (Table IV) recorded near-term pregnant females engaged in
358 courtship behaviour that is consistent with the 28 courtship events in the Maldives where 29
359 near-term pregnant females were engaged in initiation, endurance and evasion behaviours.
360 The online event number nine (Table IV) also documented initiation behaviour for *M. birostris*,
361 which means the complete sequence of courtship and mating behaviour for both species has
362 now been observed and documented. All other online courtship and mating behaviour
363 recorded in Table IV is consistent with observations recorded in the field in the Maldives in
364 this study.

365

366

DISCUSSION

367 For the first time a detailed photographic record of manta ray courtship and reproductive
368 behaviour is presented, with the observations collated here adding significant new detail to
369 previous reports in the scientific literature. Seven distinct stages to the courtship and mating
370 process in manta rays are identified, which occur within both species (Fig. 8).

371 Our study largely agrees with both Yano et al. (1999) and Marshall & Bennett (2010), but
372 advances their work by more accurately defining manta ray courtship and mating and by
373 describing a new stage (initiation). We also consider that Marshall and Bennett's sub-division
374 of 'turning and flipping' and 'evasion or avoidance' should be combined into a single category
375 because turning and flipping is the action taken by females trying to evade or avoid males.

376 Although male manta rays appear to initiate courtship, it is possible that females could
377 trigger courtship by releasing olfaction-mediated pheromones indicating readiness to mate
378 (Johnson & Nelson, 1978). This could explain the close following and courtship train behaviour
379 observed in manta rays and other elasmobranchs (Klimley, 1980; Tricas, 1980; Luer & Gilbert,
380 1985; Gordon, 1993), although no experimental evidence is available to support this
381 hypothesis (for review also see Demski, 1991). By contrast, the use of sex pheromones to
382 attract mates in the wider animal kingdom is well documented (Wyatt, 2003; Hurst, 2005).

383 Several compelling accounts of proposed olfaction-mediated sex attraction in black-tip
384 reef sharks *Carcharhinus melanopterus* have been reported from French Polynesia (Johnson
385 & Nelson, 1978). One of these accounts involved one shark tracking down another (which was
386 initially out of view), then following it closely with the snout directed towards the leader's
387 vent. Very similar behaviour in manta rays was also observed in French Polynesia during a
388 courtship event involving a near-term pregnant female *M. alfredi* and three males (M. de
389 Rosemont, pers. comm.). The four manta rays were initially engaged in a courtship train above
390 a cleaning station, but after several minutes of observations the fast-moving manta rays
391 moved out of view. However, a few minutes later the female returned, now alone, swimming
392 at above average speed along the reef in a straight line. The female continued on the same
393 course until reaching the cleaning station, at which point she changed course and headed into
394 deeper water away from the reef, disappearing from view again within 15 seconds. During

395 the next 60 seconds, all three of the males which had been following the female returned one
396 by one. Travelling in the same direction as the female, they swam along the reef in a zig-zag
397 motion with cephalic fins unfurled. Upon reaching the point where the female changed course
398 each of the males appeared to sense the direction in which she had departed as all altered
399 course to head seaward at precisely the same point she did. Similar behaviour by male *M.*
400 *alfredi* was observed during this study in the Maldives, although the event described above
401 provides the strongest circumstantial evidence to support the hypothesis that olfaction-
402 mediated cues are important in manta ray courtship and mating.

403 Further supporting the use of olfaction-mediated cues, a study on captive sandtiger sharks
404 *Carcharias taurus* by Gordon (1993) suggested the observed action of flaring and cupping of
405 the pelvic fins by females during courtship may serve as a pumping action, excreting a
406 chemical stimulant (pheromone) into the water, attracting nearby males. During courtship
407 event 39 in this study (discussed above), the pursued female excreted an almost clear liquid
408 into the water from her cloacal opening during the courtship train, repeatedly everting her
409 intestine in the process to pump the discharge into the water (Clark et al., 2007) (Fig. 9a). This
410 was quite unlike the cloudy mass of reddish-brown material which manta rays produce when
411 defecating (Fig. 9b) (pers. obs.). After the female released the clear liquid, her pursuing males'
412 sped towards her and attempted to position themselves closely behind her. In response she
413 then exhibited evasive behaviour.

414 During courtship trains the female's cephalic fins usually remain tightly rolled up, while the
415 males are usually unfurled. These modified head-fins are primarily used by manta rays during
416 feeding where they funnel planktonic food into their mouths (Paig-Tran et al., 2013; pers.
417 obs.). When manta rays are 'cruising' these fins are curled up, presumably to improve
418 hydrodynamic efficiency. Manta rays have two small nostrils, the outer openings of which are

419 situated on the upper jaw either side of their mouth. The nostrils are aligned so that while the
420 manta ray swims forward water flows into them, passing over sensory folds, allowing the
421 manta rays, like other elasmobranchs, to detect tiny concentrations of dissolved chemicals
422 (Theisen et al., 1986; Zeiske et al., 1987). Thus, if female manta rays release sex pheromones
423 during courtship trains, following male manta rays could maximise their sensitivity by
424 unfurling their cephalic fins to increase water flow to their nostrils.

425 The endurance stage of manta ray courtship consisted on average of a 3:1 (SE \pm 0.2, n =
426 168) ratio of males to females, rising to 5:1 (SE \pm 0.7, n = 40) if the courtship train escalated
427 to the evasion stage. Female manta rays and elasmobranchs in general invest heavily in their
428 offspring, while males invest little (Conrath & Musick, 2012). This means that females are
429 more likely to be selective in mate choice (Trivers, 1972; Bleu et al., 2012), thereby driving
430 contest competition among males (Cox & Le Boeuf, 1977), and explaining the female evasive
431 behaviour observed in manta rays and other elasmobranchs (Whitney et al., 2004; Pratt &
432 Carrier, 2005; Deakos, 2011). In manta rays, females can engage in multiple courtships trains
433 and determine their speed, duration and direction, which can last for hours and may not
434 result in copulation. Indeed, given that 12% (n=29) of female *M. alfredi* observed engaging in
435 courtship during this study were near-term pregnant at the time, females appear to regularly
436 engage in courtship activity before they are ready to copulate. Similar courtship behaviour by
437 near-term pregnant females from four other mobulid species (*M. birostris*, *M. mobular*, *M.*
438 *kuhlii* and *M. tarapacana*) has also been observed and appears to be a common reproductive
439 strategy employed by this family of rays (Stevens, 2016; Duffy & Tindale 2018; pers. obs.).
440 However, the fact that this study found regular occurrence of fresh mating wounds on
441 females that were not visibly pregnant but engaged in courtship trains suggests that multiple
442 matings as a result of multiple courtship events do occur, and are not uncommon.

443 A courtship train may on occasion also involve two females. During these events the lead
444 female is usually followed closely by the second which appears to be deliberately following
445 her, while males trail behind. It is unclear why this behaviour occurs, but if multiple sexually
446 receptive females are present in an area, a passing courtship train may attract additional
447 females, as the train of males provides a ready source of potential suitors for the joining
448 female. Furthermore, the presence of two females engaged in a single courtship train doubles
449 the chances of each male successfully copulating, which potentially should attract more
450 males. Ninety percent of the courtship events recorded during this study occurred at cleaning
451 stations, raising the possibility that they may also function as leks for manta rays (Stevens,
452 2016). These sites appear to create a focal point for courting animals, where individuals can
453 join passing courtship trains to assess or compete for prospective mates (Beehler & Foster,
454 1988).

455 Tonic immobility is known from many ray and shark species and may help induce females
456 to copulate and reduce risk of injury during copulation (Whitman et al., 1986; Henningsen,
457 1994). Manta rays are sensitive to touch (pers. obs.) and it is possible that tactile stimulation
458 serves as a way for males to pacify a female and trigger the onset of copulation, during which
459 a female enters a passive, almost hypnotic state. The dorsal surface of manta rays is covered
460 by a layer of mucus which contains dark pigmentation, creating darker shading where the
461 mucus layer is thickest. The layer is easily rubbed off through light contact (pers. obs.). During
462 courtship the males' unfurled cephalic fins rub the back of the female's head or pectoral fin,
463 revealing a lighter skin tone underneath. These marks quickly darken and the natural skin
464 pigmentation returns within a few weeks, unlike the permanent scars on the dorsal surface
465 of the females' pectoral fin tips which can arise from mating.

466 At the onset of pre-copulation positioning, whether tactile stimulation plays a role or not,
467 something causes the female to reduce her swimming speed and cease evasive behaviour,
468 allowing the male to grasp her pectoral fin. As the majority of copulation events observed
469 consisted of just a single male and female, the lengthy duration of courtship trains may
470 constitute a form of control by females to selectively reduce competing males until only the
471 most persistent remains, similar to the heat runs exhibited by humpback whales *M.*
472 *novaeangliae*, where the principal escort (male) attempts to hold his position next to the
473 female throughout her late pregnancy (Baker & Herman, 1984; Herman et al., 2007). In this
474 way females may ensure only the fittest males are selected as a mate.

475 During our study, near-term pregnant females were regularly involved in courtship trains,
476 suggesting that females are likely to mate soon after giving birth. Indeed, fresh mating
477 wounds were recorded on females soon after parturition, although any subsequent gestation
478 often appears to be delayed for many months or even years in the wild (Stevens, 2016). These
479 field observations are supported by the mating behaviour of a female *M. alfredi* held in
480 captivity in Okinawa Churaumi Aquarium, Japan (Okinawa Churaumi Aquarium, 2010). From
481 four consecutive pregnancies this individual gave birth to a single pup then copulated within
482 hours of parturition. To date, these are the only observations of any manta ray giving birth.

483 Protracted courtship may also increase reproductive success in other ways. Females that
484 allow copulation while multiple males are still engaged in courtship activity with her run the
485 risk of injury through collision with the reef. The presence of multiple males during the later
486 stages of courtship is also likely to reduce the chance of a successful copulation as competing
487 males prevent each other from successfully positioning themselves for penetration. Previous
488 mating experience of a female may also play a role in the timing of the pre-copulation
489 positioning trigger.

490 Post-copulation holding behaviour was observed in six of the seven previously described
491 manta ray mating events (Yano et al., 1999; Marshall & Bennett, 2010). However, of the four
492 documented copulation events in this study, no post-copulation holding was observed, with
493 separation commencing simultaneously upon cessation of copulation. It is unclear what
494 benefit, or significance, may be derived from this post-copulatory behaviour, therefore future
495 studies will need to determine if it warrants the current stage categorisation.

496 In summary, this study confirms that both *M. alfredi* and *M. birostris* appear to engage in
497 the same elaborate courtship rituals, exhibiting similar behaviours during all stages of the
498 courtship and mating process. These courtship rituals are most prevalent at cleaning stations
499 in *M. alfredi*, which may also function as lekking sites (Stevens, 2016). The study also suggests
500 female manta rays invest heavily in mate choice, thereby shaping their reproductive
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502

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515

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REFERENCES

- 517 Anderson, R. C., Adam, M. S. & Goes, J. I. (2011a). From monsoons to mantas: seasonal
518 distribution of *Manta alfredi* in the Maldives. *Fisheries Oceanography* **20**, 104–113.
- 519 Anderson, R. C., Adam, M. S., Kitchen-Wheeler, A.-M. & Stevens, G. M. W. (2011b). Extent and
520 economic value of manta ray watching in Maldives. *Tourism in Marine Environments* **7**,
521 15–27.
- 522 Baker, C. S. & Herman, L. M. (1984). Aggressive behavior between humpback whales
523 *Megaptera novaeangliae* wintering in Hawaiian waters. *Canadian Journal of Zoology* **62**,
524 1922–1937.
- 525 Bancroft, E. N. (1829). On the fish known in Jamaica as the sea-devil. In N. A. Vigors, ed. *The*
526 *Zoological Journal* **4**, 444–457.
- 527 Beebe, W. & Tee-Van, J. (1941). Eastern Pacific expeditions of the New York Zoological
528 Society. XXVIII Fishes from the tropical eastern Pacific. Part 3: rays, mantas, and
529 chimaeras. *Zoologica* **26**, 245–278.
- 530 Beehler, B. M. & Foster, M. S. (1988). Hotshots, hotspots, and female preference in the
531 organization of lek mating systems. *American Society of Naturalists* **131**, 203–219.
- 532 Bigelow, H. B. & Schroeder, W. C. (1953). Sawfish, guitarfish, skates and rays. In *Fishes of the*
533 *Western North Atlantic* **2**, 508–514.
- 534 Bleu, J., Bessa-Gomes, C. & Laloi, D. (2012). Evolution of female choosiness and mating
535 frequency: effects of mating cost, density and sex ratio. *Animal Behaviour* **83**, 131–136.
- 536 Braun, C. D., Skomal, G. B., Thorrold, S. R. & Berumen, M. L. (2014). Diving behavior of the
537 reef manta ray links coral reefs with adjacent deep pelagic habitats. *PLoS ONE* **9**, 1–8.
- 538 Byers, J. A., Wiseman, P. A., Jones, L. & Roffe, T. J. (2005). A large cost of female mate sampling
539 in pronghorn. *The American Naturalist* **166**, 661–668.
- 540 Castro, J. I. (2009). Observations on the reproductive cycles of some viviparous North
541 American sharks. *Aqua, International Journal of Ichthyology* **15**, 205–222.
- 542 Catry, P., Phillips, R. A., Phalan, B. & Croxall, J. P. (2006). Senescence effects in an extremely
543 long-lived bird: the grey-headed albatross *Thalassarche chrysostoma*. *Proceedings of the*
544 *Royal Society B: Biological Sciences* **273**, 1625–30.
- 545 Clark, T. B., Papastamatiou, Y. P. & Meyer, C. G. (2007). Intestinal eversion in a free-ranging
546 manta ray *Manta birostris*. *Coral Reefs* **27**, 61–61.
- 547 Coles, R. J. (1916). Article XXXIII. Natural history notes on the devil fish, *Manta birostris*
548 (Walbaum) and *Mobula olfersi* (Müller). *Bulletin of the American Museum of Natural*
549 *History* **35**, 649–657.
- 550 Colonello, J. H., Lucifora, L. O. & Massa, A. M. (2006). Reproduction of the angular angel shark
551 *Squatina guggenheim*: geographic differences, reproductive cycle, and sexual
552 dimorphism. *ICES Journal of Marine Science* **64**, 131–140.
- 553 Compagno, L. J. V. & Last, P. R. (1999). Mobulidae: devil rays. In K. E. Carpenter & V. H. Niem,
554 eds. *The Living Marine Resources of the Western Central Pacific*. 1524–1529.

555 Conrath, C. L. & Musick, J. A. (2012). Reproductive biology of elasmobranchs. In J. C. Carrier,
556 J. A. Musick, & M. R. Heithaus, eds. *Biology of Sharks and Their Relatives*. 291–312.

557 Côté, I. M. (2000). Evolution and ecology of cleaning symbioses in the sea. *Oceanography and*
558 *Marine Biology* **38**, 311–355.

559 Couturier, L. I. E., Marshall, A. D., Jaine, F. R. A., Kashiwagi, T., Pierce, S. J., Townsend, K. A.,
560 Weeks, S. J., Bennett, M. B. & Richardson, A. J. (2012). Biology, ecology and conservation
561 of the Mobulidae. *Journal of Fish Biology* **80**, 1075–1119.

562 Cox, C. R. & Le Boeuf, B. J. (1977). Female incitation of male competition: a mechanism in
563 sexual selection. *The American Naturalist* **111**, 317–335.

564 Cree, A., Guillette, L. J. & Guillette, Jr, L. J. (1995). Biennial reproduction with a fourteen-
565 month pregnancy in the gecko *Hoplodactylus maculatus* from Southern New Zealand.
566 *Journal of Herpetology* **29**, 163–173.

567 Deakos, M. H. (2011). The reproductive ecology of resident manta rays *Manta alfredi* off
568 Maui, Hawaii, with an emphasis on body size. *Environmental Biology of Fishes* **94**, 1–14.

569 Demski, L. (1991). Elasmobranch reproductive behavior: implications for captive breeding.
570 *Journal of Aquaculture and Aquatic Sciences* **5**, 84–95.

571 Dewar, H., Mous, P., Domeier, M., Muljadi, A., Pet, J. & Whitty, J. (2008). Movements and site
572 fidelity of the giant manta ray *Manta birostris* in the Komodo Marine Park, Indonesia.
573 *Marine Biology* **155**, 121–133.

574 Dodd, J. M. (1983). Reproduction in cartilaginous fishes Chondrichthyes. In W. S. Har, D. J.
575 Randall, & E. M. Donaldson, eds. *Fish Physiology* **9**, 31–95.

576 Duffy, C. A. J. & Tindale, S. C. (2018). First observation of the courtship behaviour of the giant
577 devil ray *Mobula mobular* (Myliobatiformes: Mobulidae). *New Zealand Journal of*
578 *Zoology* **45**, 1–8. doi.org/10.1080/03014223.2017.1410850.

579 Dulvy, N. K. & Reynolds, J. D. (1997). Evolutionary transitions among egg-laying, live-bearing
580 and maternal inputs in sharks and rays. *Proceedings of the Royal Society B: Biological*
581 *Sciences* **264**, 1309–1315.

582 Fedigan, L. M. & Rose, L. M. (1995). Interbirth interval variation in three sympatric species of
583 neotropical monkey. *American Journal of Primatology* **37**, 9–14.

584 Gilmore, R. G. (1993). Reproductive biology of lamnoid sharks. *Environmental Biology of*
585 *Fishes* **38**, 95–114.

586 Gordon, I. (1993). Pre-copulatory behavior of captive sandtiger sharks *Carcharias taurus*.
587 *Environmental Biology of Fishes* **38**, 159–164.

588 Henningsen, A. D. (1994). Tonic immobility in 12 elasmobranchs: use as an aid in captive
589 husbandry. *Zoo Biology* **13**, 325–332.

590 Herman, E. Y. K., Herman, L. M., Pack, A. A., Marshall, G., Shepard, M. C. & Bakhtiari, M.
591 (2007). When whales collide: crittercam offers insight into the competitive behavior of
592 humpback whales on their Hawaiian wintering grounds. *Marine Technology Society*
593 *Journal* **41**, 35–43.

594 Hurst, J. L. (2005). Scent marking and social communication. In P. K. McGregor, ed. *Animal*
595 *Communication Networks*. 219–243.

596 Ibarregüengoytia, N. R. & Cussac, V. E. (1996). Reproductive biology of the viviparous lizard
597 *Liolaemus pictus* Tropicuridae: biennial female reproductive cycle? *Herpetological*
598 *Journal* **6**, 137–143.

599 Jaine, F. R. A., Couturier, L. I. E., Weeks, S. J., Townsend, K. A., Bennett, M. B., Fiora, K. &
600 Richardson, A. J. (2012). When giants turn up: sighting trends, environmental influences
601 and habitat use of the manta ray *Manta alfredi* at a Coral Reef. *PLoS ONE*, **7**, 1–10. Jaine,

602 F. R. A., Rohner, C. A., Weeks, S. J., Couturier, L. I. E., Bennett, M. B., Townsend, K. A. &
603 Richardson, A. J. (2014). Movements and habitat use of reef manta rays off eastern
604 Australia: offshore excursions, deep diving and eddy affinity revealed by satellite
605 telemetry. *Marine Ecology Progress Series* **510**, 73–86.

606 Johnson, R. H. & Nelson, D. R. (1978). Copulation and possible olfaction-mediated pair
607 formation in two species of carcharhinid sharks. *Copeia* **1978**, 539–542.

608 Kashiwagi, T., Marshall, A. D., Bennett, M. B. & Ovenden, J. R. (2011). Habitat segregation and
609 mosaic sympatry of the two species of manta ray in the Indian and Pacific Oceans: *Manta*
610 *alfredi* and *M. birostris*. *Marine Biodiversity Records* **4**, 1–8.

611 Kitchen-Wheeler, A.-M., Ari, C. & Edwards, A. J. (2011). Population estimates of Alfred mantas
612 *Manta alfredi* in central Maldives atolls: North Male, Ari and Baa. *Environmental Biology*
613 *of Fishes* **93**, 557–575.

614 Klimley, A. P. (1980). Observations of courtship and copulation in the nurse shark
615 *Ginglymostoma cirratum*. *Copeia* **1980**, 878–882.

616 Krefft, G. (1868). *Deratoptera alfredi* Prince Alfred's ray. *Illustrated Sydney News* **5**, 1–16.

617 Kucera, T. E. (1978). Social behavior and breeding system of the desert mule deer. *American*
618 *Society of Mammalogists* **59**, 463–476.

619 Lee, P. C. & Moss, C. J. (1986). Early maternal investment in male and female African elephant
620 calves. *Behavioral Ecology and Sociobiology* **18**, 353–361.

621 Losey Jr, G. S. (1972). The ecological importance of cleaning symbiosis. *Copeia* **1972**, 820–833.

622 Luer, C. A. & Gilbert, P. W. (1985). Mating behavior, egg deposition, incubation period, and
623 hatching in the clearnose skate *Raja eglanteria* *Environmental Biology of Fishes* **13**, 161–
624 171.

625 Mann, J., Connor, R. C., Barre, L. M. & Heithaus, M. R. (2000). Female reproductive success in
626 bottlenose dolphins *Tursiops* sp.: life history, habitat, provisioning, and group-size
627 effects. *Behavioral Ecology* **11**, 210–219.

628 Marshall, A. D., Compagno, L. J. V. & Bennett, M. B. (2009). Redescription of the genus *Manta*
629 with resurrection of *Manta alfredi* (Krefft, 1868) (Chondrichthyes; Myliobatoidei;
630 Mobulidae). *Zootaxa* **2301**, 1–28.

631 Marshall, A. D. & Bennett, M. B. (2010). Reproductive ecology of the reef manta ray *Manta*
632 *alfredi* in southern Mozambique. *Journal of Fish Biology* **77**, 169–90.

633 Marshall, A. D. & Pierce, S. J. (2012). The use and abuse of photographic identification in
634 sharks and rays. *Journal of Fish Biology* **80**, 1361–1379.

635 Mysterud, A., Langvatn, R. & Stenseth, N. C. (2004). Patterns of reproductive effort in male
636 ungulates. *Journal of Zoology* 209–215.

637 van Noordwijk, M. A. & van Schaik, C. P. (2005). Development of ecological competence in
638 Sumatran orangutans. *American Journal of Physical Anthropology* **127**, 79–94.

639 O'Shea, O. R., Kingsford, M. J. & Seymour, J. (2010). Tide-related periodicity of manta rays and
640 sharks to cleaning stations on a coral reef. *Marine and Freshwater Research* **61**, 65–73.

641 Paig-Tran, E. W. M., Kleinteich, T. & Summers, A. P. (2013). The filter pads and filtration
642 mechanisms of the devil rays: variation at macro and microscopic scales. *Journal of*
643 *Morphology* **274**, 1026–43.

644 Pratt, H. L. & Carrier, J. C. (2001). A review of elasmobranch reproductive behavior with a case
645 study on the nurse shark *Ginglymostoma cirratum*. *Environmental Biology of Fishes* **60**,
646 157–188.

647 Pratt, H. L. & Carrier, J. C. (2005). Elasmobranch courtship and mating behavior. In W. C.
648 Hamlett, ed. *Reproductive Biology and Phylogeny of Chondrichthyes*. 129–170.

649 Rafinesque-Schmaltz, C. S. (1810). *Indice d'ittiologia siciliana ossia catalogo metodico dei*
650 *nomi latini, italiani e siciliani dei pesci, che si rinvencono in Sicilia*, Messina.

651 Robinson, M. R., Mar, K. U. & Lummaa, V. (2012). Senescence and age-specific trade-offs
652 between reproduction and survival in female Asian elephants. *Ecology Letters* **15**, 260–
653 266.

654 Rubin, R. (2002). Manta rays: not all black and white. *Shark Focus*, **15**, 4–5.

655 Sever, D. M., Ryan, T. J., Morris, T., Patton, D. & Swafford, S. (2000). Ultrastructure of the
656 reproductive system of the black swamp snake *Seminatrix pygaea*. II. Annual oviducal
657 cycle. *Journal of Morphology* **245**, 146–160.

658 Spitz, S. S., Herman, L. M., Pack, A. A. & Deakos, M. H. (2002). The relation of body size of
659 male humpback whales to their social roles on the Hawaiian winter grounds. *Canadian*
660 *Journal of Zoology* **80**, 1938–1947.

661 Stevens, G. M. W. (2016). Conservation and population ecology of manta rays in the Maldives.
662 PhD Thesis. University of York, York, U.K.

663 Stewart, J. D., Hoyos-Padilla, E. M., Kumli, K. R. & Rubin, R. D. (2016a). Deep-water feeding
664 and behavioral plasticity in *Manta birostris* revealed by archival tags and submersible
665 observations. *Zoology* **119**, 406–413.

666 Stewart, J. D., Beale, C. S., Fernando, D., Sianipar, A. B., Burton, R. S., Semmens, B. X. & Aburto-
667 Oropeza, O. (2016b). Spatial ecology and conservation of *Manta birostris* in the Indo-
668 Pacific. *Biological Conservation* **200**, 178–183.

669 Theisen, B., Zeiske, E. & Breucker, H. (1986). Functional morphology of the olfactory organs
670 in the spiny dogfish *Squalus acanthias* and the small-spotted catshark *Scyliorhinus*
671 *canicula*. *Acta Zoologica* **67**, 73–86.

672 Tricas, T. C. (1980). Courtship and mating-related behaviors in myliobatid rays. *Copeia* **1980**,
673 553–556.

674 Trinnie, F. I., Walker, T. I., Jones, P. L. & Laurenson, L. J. (2012). Biennial reproductive cycle in
675 an extensive matrotrophic viviparous batoid: the sandyback stingaree *Urolophus*
676 *bucculentus* from south-eastern Australia. *Journal of Fish Biology* **80**, 1267–1291.

677 Trivers, R. L. (1972). Parental investment and sexual selection. In B. Campbell, ed. *Sexual*
678 *selection and the descent of man*. 136–179.

679 Tyack, P. & Whitehead, H. (1982). Male competition in large groups of wintering humpback
680 whales. *Behaviour* **83**, 132–154.

681 Uchida, S., Toda, M., Kamei, Y., Hoening, J. & Gruber, S. (1990). Reproduction of elasmobranchs
682 in captivity. In H. L. Pratt, S. H. Gruber, & T. Taniuchi, eds. *Elasmobranchs as Living*
683 *Resources: Advances in the biology, ecology, systematics, and the status of the fisheries*.
684 211–237.

685 Vos, A. De, Brokx, P. & Geist, V. (1967). A review of social behavior of the North American
686 cervids during the reproductive period. *American Midland Naturalist* **77**, 390–417.

687 Walbaum, J. J. (1792). Grypeswaldiae. In *Petri Artedi Sueci Genera Piscium*. 535.

688 White, W. T., Corrigan, S., Yang, L. E. I., Henderson, A. C., Bazinet, A. L., Swofford, D. L. &
689 Naylor, G. J. P. (2017). Phylogeny of the manta and devilrays (Chondrichthyes: M
690 obulidae), with an updated taxonomic arrangement for the family. *Zoological Journal of*
691 *the Linnean Society* **182**, 1–26. doi.org/10.1093/zoolinnean/zlx018.

692 Whitman, P. A., Marshall, J. A. & Keller, Jr., E. C. (1986). Tonic immobility in the smooth dogfish
693 shark *Mustelus canis* (Pisces, Carcharhinidae). *Copeia* **1986**, 829–832.

694 Whitney, N. M. & Crow, G. L. (2006). Reproductive biology of the tiger shark *Galeocerdo cuvier*
695 in Hawaii. *Marine Biology* **151**, 63–70.

- 696 Whitney, N. M., Pratt, H. L. & Carrier, J. C. (2004). Group courtship, mating behaviour and
697 siphon sac function in the whitetip reef shark *Triaenodon obesus*. *Animal Behaviour* **68**,
698 1435–1442.
- 699 Wolff, J. O. (1998). Breeding strategies, mate choice, and reproductive success in American
700 bison. *Oikos* **83**, 529–544.
- 701 Wourms, J. P. (1977). Reproduction and development in chondrichthyan fishes. *American*
702 *Zoologist* **17**, 379–410.
- 703 Wourms, J. P. & Demski, L. S. (1993). The reproduction and development of sharks, skates,
704 rays and ratfishes: introduction, history, overview, and future prospects. *Environmental*
705 *Biology of Fishes* **38**, 7–21.
- 706 Wyatt, T. D. (2003). Pheromones and animal behaviour: communication by smell and taste.
707 1-391.
- 708 Yano, K., Sato, F. & Takahashi, T. (1999). Observations of mating behavior of the manta ray
709 *Manta birostris* at the Ogasawara Islands, Japan. *Ichthyological Research* **46**, 289–296.
- 710 Zeiske, E., Theisen, B. & Gruber, S. H. (1987). Functional morphology of the olfactory organ of
711 two carcharhinid shark species. *Canadian Journal of Zoology* **65**, 2406–2412.
- 712
- 713

714

ELECTRONIC REFERENCES

- 715 Marshall, A. D., Kashiwagi, T., Bennett, M. B., Deakos, M. H., Stevens, G. M. W., McGregor, F.,
716 Clark, T., Ishihara, H. & Sato, K. (2011a). *Manta alfredi*. In *IUCN Red List of Threatened*
717 *Species* Version 2011. Available at <http://www.iucnredlist.org/details/195459/0> (last
718 accessed 20 November 2017).
- 719 Marshall, A. D., Bennett, M. B., Kodja, G., Hinojosa-Alvarez, S., Galvan-Magana, F., Harding,
720 M., Stevens, G. M. W. & Kashiwagi, T. (2011b). *Manta birostris*. In *IUCN Red List of*
721 *Threatened Species* Version 2011. Available at
722 <http://www.iucnredlist.org/details/198921/0> (last accessed 20 November 2017).
- 723 Okinawa Churaumi Aquarium (2010). We have just recently had our 4th successful manta ray
724 (*Manta birostris*) birth in captivity at Okinawa Churaumi Aquarium In *News release –*
725 *Okinawa Churaumi Aquarium*. Available at [http://oki-](http://oki-churaumi.jp/info/ennews/archives/)
726 [churaumi.jp/info/ennews/archives/](http://oki-churaumi.jp/info/ennews/archives/) (accessed 9 September 2011).